Gonadotropin Releasing Hormone (GnRH) Agonist Test in Disorders of Puberty

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GnRH Agonist Test in Disorders of Puberty.
Outline.

• Overview
• The nature of the problem
• Background endocrinology
• Background of this study: antecedent studies
• Adverse events of leuprolide
• Protocol #13472A
• Summary
Overview: GnRH Agonists are Promising Diagnostics

- Delayed Puberty (esp. a diagnostic problem in boys)
  - Constitutional Delay of Puberty
    (CDP, “an extreme variant of normal”) vs
    Gonadotropin Deficiency (GnD)
- Premature (Precocious) Puberty (esp. in girls)
  - Idiopathic True / Central Precocious Puberty
    (CPP, “an extreme variant of normal”) vs
  - Normal Prepubertal and
  - Premature Pseudo-Puberty (diverse types)
- Need for Normative Data on Healthy Prepubertal & Early Pubertal Children

CDP & Idiopathic CPP: “Extreme Variants of Normal”

Conceptual Definition of Premature & Delayed Puberty

2.5%: Premature Puberty
2.5%: Delayed Puberty

CDP and CPP are source of most “normal” data
Problem 1: Differentiating Constitutional Delay of Puberty (CDP) from Gonadotropin Deficiency (GnD)

- Delay mostly in boys
- CDP boys develop increasingly poor self-image after 14 years
- “Grow out of it”
- Cause: “nl variant”
- W/U: minimal
- Rx: reassurance ± 6 month T boost
- Contrasts with GnD

Longitudinal F/U of Boy with CDP (years)


Problem 2: Differentiating Idiopathic CPP from Normal Variants & Other Pseudo-Precocity. I.

- Precocity predominantly in girls
- CPP scary for child & parents
  » moody
  » periods?
  » early growth arrest
- Over > 90% “nl stage just early”
- W/U: minimal (brain MRI when rapidly progressive)
- Rx: reassurance ± GnRH ag chronically until ~11 yo
- Pseudo-precocity may be normal variant or due to neoplasm, etc
Problem 2: Differentiating Idiopathic CPP from Normal Variants. II. The Problem of Early Thelarche

“Yesterday’s Precocious Puberty is Norm Today”
- N Y Times, December 7, 1999

“Doubters Fault Theory Finding Earlier Puberty”
- N Y Times, February 20, 2001

“2 Endocrinology Groups Raise Doubt on Earlier Onset of Girls' Puberty”
- N Y Times, March 3, 2001

• While there is some evidence that breast development may be occurring 1-2 years earlier, esp. in the obese, age of menarche is unchanged--is this true puberty?

BACKGROUND ENDOCRINOLOGY OF PUBERTY

- Subclinical neonatal mini-puberty
- CNS inhibition begins
- Pulsatile GnRH during sleep
- Adrenarche begins
- Pituitary sensitivity increases
- Gonadal sensitivity increases
- Amplification

CNS Inhibition of GnRH Pulse Generator
Amplification

Gonadal sensitivity increases during sleep

Pulsatile GnRH

Pituitary sensitivity increases

Amplification

GnRH:
Gonadotropin Releasing Hormone

Gonadotropins
- LH
- FSH

LH -->
Estradiol / Testosterone

FSH -->
Egg / sperm

• Rationale: response to GnRH/ag reflects previous exposure to GnRH

BACKGROUND OF GnRH AGONIST TESTING. I.

• 1977: Nobel Prize for Discovery of GnRH
• ‘80’s: Desensitizing Effect of Chronic GnRH Agonist Analogs -> Chronic GnRH Agonist Rx for CPP (ODP)
• 1985: PI GCRC Studies Under Expanded Syntex IND for Nafarelin Treatment of Central Precocious Puberty
• Examined patients’ hormonal responses to 1st dose of nafarelin (out of my interest in the acute response being a potentially useful diagnostic test)
• Subjects: CPP girls starting nafarelin (GnRHa) Rx

• Compared GnRH 3 hr infusion test to a nafarelin test of pituitary-gonadal axis

• Results: LH & FSH responses to GnRHa greater & more prolonged than to GnRH -- estradiol response

Rosenfield, et al JCEM 1986
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• 1985: PI GCRC Studies Under Expanded Syntex IND for Nafarelin Treatment of Central Precocious Puberty
• Comparison of CPP’s hormonal response to nafarelin dose #1 by injection to natural GnRH infusion test » showed acute agonistic effect on LH-FSH-E2
• 13472A precursor protocols were pilot studies to explore diagnostic potential of nafarelin in children with known or suspected disorders of puberty (mostly CDP or CPP)

**Girls with variations of normal pubertal development**

Nafarelin tests in girls at various pubertal stage

**BACKGROUND:** GnD vs CDP in prepubertal boys

**Sleep Tests:**

Provisional sleep test discriminatory criterion: \( \Delta LH \geq 0.35 \text{ U/L} \) separates 18/19

Ghai, et al JCEM 1995

**GnRH Agonist (Nafarelin) Tests:**

\( \Delta LH \geq 4.8 \text{ U/L} \) discriminates 19/20

Ghai, et al JCEM 1995
BACKGROUND OF GnRH AGONIST TESTING. II.

• 1992-93: Syntex sold out & Searle not interested
  PI obtained IND #40,387 (1992): ‘93 nafarelin -> leuprolide
  • Several GCRC protocols with co-investigators
    » Hyperandrogenism in adult women and children
    » Disorders of puberty (CDP, CPP, etc)
    » TAP 1 yr bridge funding --> no further support
  • FD-R-001012 (1994, ODP)
    » Adult dose-response study & comparison to naf/GnRH
    » GnD vs CDP
  • FD-R-001473 (1997, OPD)
    » Adult GnD trial of intermittent GnRHag Rx

IND #60,003 (2000): leuprolide
• RO1-HD-39267 (‘01): hyperandrogenism (child & adult)

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Dose-response study of leuprolide (Lupron®) in adults + comparison to GnRH (Factrel®) in men & historical nafarelin data

Results
• Lupron 10 mcg/kg similar to naf in LH & sex steroid stimulation
• Lupron less potent FSH stimulant

--Rosenfield, et al. JCEM 1996
Snags!
1. RIA results unlike monoclonal (“3rd gen.”) assay for LH

Why?
- Microheterogeneity of LH
- Our RIA had enhanced specificity for bioactive LH, but incomp.
- Delfia ß-subunit specific

2. Alarmed freezer failure: lost samples before 2001

Meanwhile--
other sex-specific potential end-points discovered in pubertal variants

Boys (n=11) & girls (n=7) w. BA >7.8 y
BACKGROUND OF GnRH AGONIST TESTING. III. Summary to Date.

- Leuprolide not quite same as nafarelin re FSH stim
- Can’t go back to discriminatory RIA
- Considerable promising preliminary data in children from multiple peer-reviewed studies at many levels (GCRC/site visits, FDA- and R01-reviews)
- Starting over

ADVERSE EVENTS OF LEUPROLIDE TEST (1 Injx)


- No Serious Adverse Events
- Anticipated Side Effects
  - Children under 18 years of age (n = 332)
    » IV-related: 3 (soreness, hematoma) -> one withdrew
    » local allergic reaction (rash), transient: 1
  - Adults (n = 245)
    » IV-related: 1
    » local allergic reaction (rash), transient: 1
    » Hormone-related side effects (post-study): 14
      – menstrual pattern change: 3 (1 pre-existing PCOS)
      – PMS symptoms mood, cramps, h/a: 11
      (1 of the 3 males improved)
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ADVERSE EVENTS OF LEUPROLIDE TREATMENT

**Wide use of leuprolide long-term Rx (Depot Lupron®)**

- Children with CPP (short stature)
- Adult men with prostate cancer
- (Adult women with endometriosis, fibroids, fertility Rx)

**Side effects of long-term Rx**

- Depot -> 5-10% develop sterile abscesses at injx sites
- Hormone-related side effects
  - Menstrual irregularity
  - PMS symptoms (mood changes, swelling, h/a, etc)
  - Memory effects? (data contradictory--Yaffe, *JAMA* 2003)
  - osteopenia
- No increase in birth defects (*Jannsens 2000*)
ADVERSE EVENTS OF LEUPROLIDE ACETATE. 
Response to Adverse Public Comment (4 letters)

Mis-informed and/or related to long-term therapy

- Package insert adverse effects are those of 2 yr Rx of prostate ca
- No “black box” warning
- Human evidence for adverse effect on autoimmunity insufficient to warrant warning
- Not a hazardous drug requiring chemo precautions

Other Public Comments

- Lawson Wilkins Pediatric Endocrine Society, The Endocrine Society, The American Society for Reproductive Medicine are unconcerned about leuprolide acetate test toxicity
- LWPES notes that “leuprolide is used in the routine diagnostic testing of children to determine the initiation of puberty…highly useful…normative data are sparse”
- The Endocrine Society adds that, while determining sleep-related LH secretion is the “gold standard,” it is (potentially) “less invasive than the leuprolide test.”
PROTOCOL 13472A: 
GnRH Agonist Test in Disorders of Puberty

Hypothesis
Hormonal responses to GnRH agonist (GnRHag) test will distinguish among disorders of puberty as well as a sleep test.

Specific Aims
1. Distinguish among the causes of premature puberty:
   a. idiopathic CPP (vs. prem. thelarche) vs healthy vols
   b. gonadotropin-indep precocity (e.g., tumor) vs idiopathic CPP
2. Distinguish among the causes of delayed puberty:
   a. GnD vs CDP

CDP & Idiopathic CPP: “Extreme Variants of Normal”?

• Practice assumes that these are normal variants
• Pubertal tempo, menstrual cyclicity, and fertility in adult life typically within broad range of normal
• Familial in about half

• Evidence that a small percent of these “normal variants” may not be normal:
• Slow tempo of those starting puberty at 6-7 years
• Family history of delayed puberty in ~10-15% of GnD
• GnRH receptor SNPs nominally associated with variations in timing of puberty (Sedlmeyer, JCEM Oct ‘05)
• Mouse chromosomes 6 and 11 harbor genes that regulate pubertal timing (Krewson, Endocrinol 2004)

• Normal population data needed to avoid misclassif.
PROTOCOL #13472A: GnRH Agonist Test in Disorders of Puberty

Study Design

• Subjects: 20 per group of each sex.
  » Normal volunteers:
    – Prepubertal male, 9-13 years old
    – Prepubertal female, 8-12 years old
    – Early pubertal male, 9-15 years old
    – Early pubertal female, premenarcheal, 9-15 years old
  » Patient groups
    – CDP vs GnD
    – CPP vs gonadotropin-independent precocity and premature thelarche
**GnRH Agonist Test in Disorders of Puberty**

**Analysis of Data. I.**

**Sleep test:** significant increase LH ≥ 0.35 U/L provisionally defines puberty onset

- Normal range set at 5-95 %ile healthy volunteers
  - Secondary: 5-95%ile for CDP (boys) & CPP (girls)

**GnRH agonist (leuprolide) test:**

- Hormone response primary variables (group-specific)
  - Pituitary: LH, free alpha subunit (FAS)
  - Gonads: Sex steroid (T or E2), inhibin-B

- **Sex- and stage-specific 5-95%ile ranges set for:**
  - Normals (healthy volunteers)
  - CDP and CPP

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**GnRH Agonist Test in Disorders of Puberty**

**Analysis of Data. II.**

- **Boys** *(common dx problem is delayed puberty)*:
  - Primary comparison: GnD vs CDP *(stage-specific)*
  - Secondary: GnD vs healthy volunteers *and* CDP vs healthy volunteers
  - *Tertiary: girls*

- **Girls** *(common dx problem is premature puberty)*:
  - Primary: CPP vs prepubertal healthy volunteers
  - Secondary: CPP vs *pseudo-pubertal* groups*
    - (gonadotropin-independent precocity and premature thelarche)
  - *Tertiary: boys*

* *Power is limited for some sub-groups*
GnRH Agonist Test in Disorders of Puberty: Summary

• GnRH agonist testing is of minimal risk
• Study design straight-forward: normal vs abnormal
• Adequate statistical power for primary comparisons
• Significance for clinical care: great
  » This protocol will develop badly needed data on the hormonal responses to leuprolide in normal prepubertal and pubertal children, using commercially available state-of-the-art assays
  » It will also provide data on the diagnostic value of the test for the most common pubertal disorders